Supporting Information for:

Digital Microfluidics with Distance-Based Detection – A New Approach for Nucleic Acid Diagnostics

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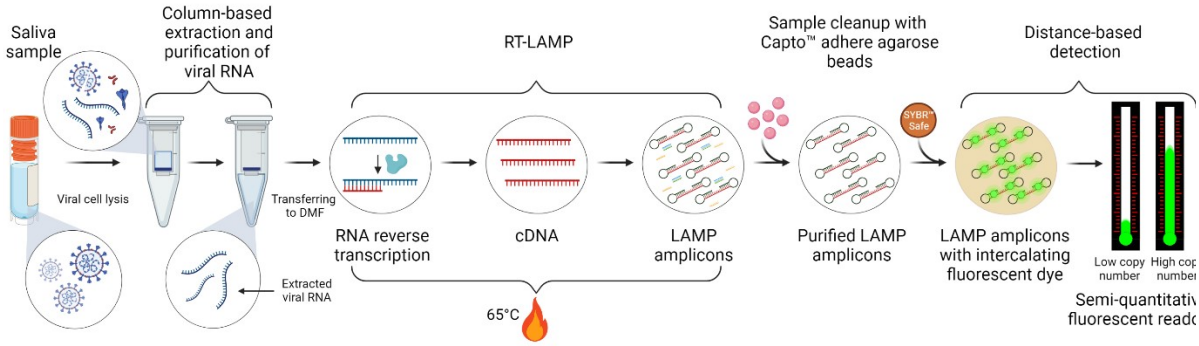

Figure S1. Schematic representation of the steps involved in the DMF-DBD assay. The saliva sample is lysed, and any viral RNA is extracted and purified using columns. The sample is then loaded onto a DMF cartridge, where the RNA is reverse transcribed to DNA and then amplified using LAMP. Capto™ adhere agarose beads are used to clean up LAMP amplicons. Then, the purified LAMP amplicons are mixed with an intercalating dye (SYBR Safe) and finally, the mixture is introduced delivered to the DBD substrate. The distance of the fluorescent signal (Green) on the DBD is used to determine the viral load in the saliva sample semi-quantitatively. The figure was created using Biorender (https://www.biorender.com/).

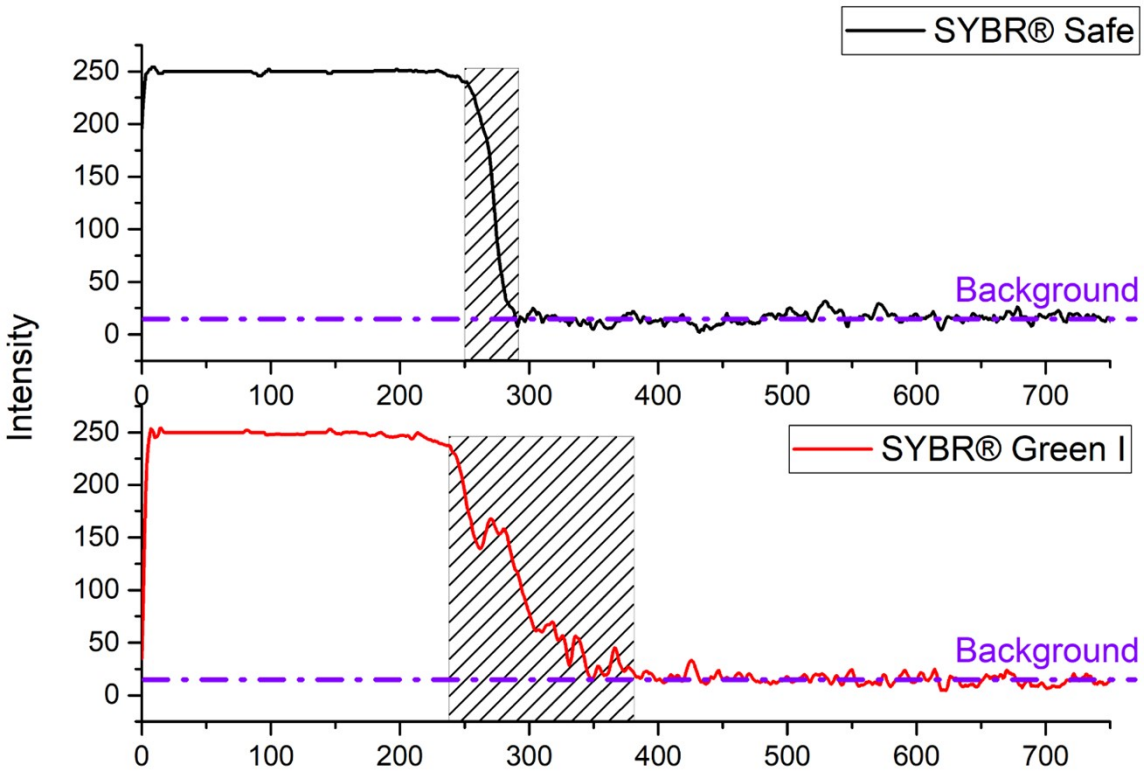

Figure S2. Representative linear plots of fluorescent intensity in DBD substrates. These data feature 100x SYBR® Safe (black, above) and SYBR® Green I (red, below) in 25 µg/mL dsDNA standard as a function of pixel number, counted from the loading zone. The 'fading front' phenomenon is illustrated in shaded boxes, indicating the distances between the last pixel with maximum intensity and the first pixel with intensity equivalent or lower than background intensity (purple dashed line).

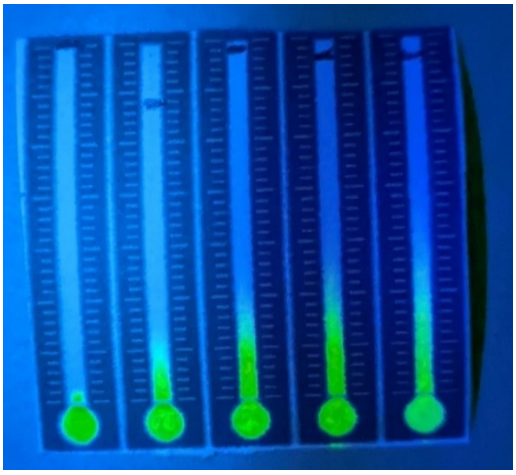

Figure S3. DBD imaging by "eye". Representative image of 0, 10, 25, 50, and 75 µg/mL (from left to right) dsDNA standard in nuclease-free water with 100x SYBR® SAFE on a DBD substrate formed from Whatman™ grade 4 paper. The image was captured using a Pixel 6 cell phone camera with fluorescent filter (485 nm) and single-band pass filter (561/14 nm) installed in front of the flashlight and camera aperture, respectively.

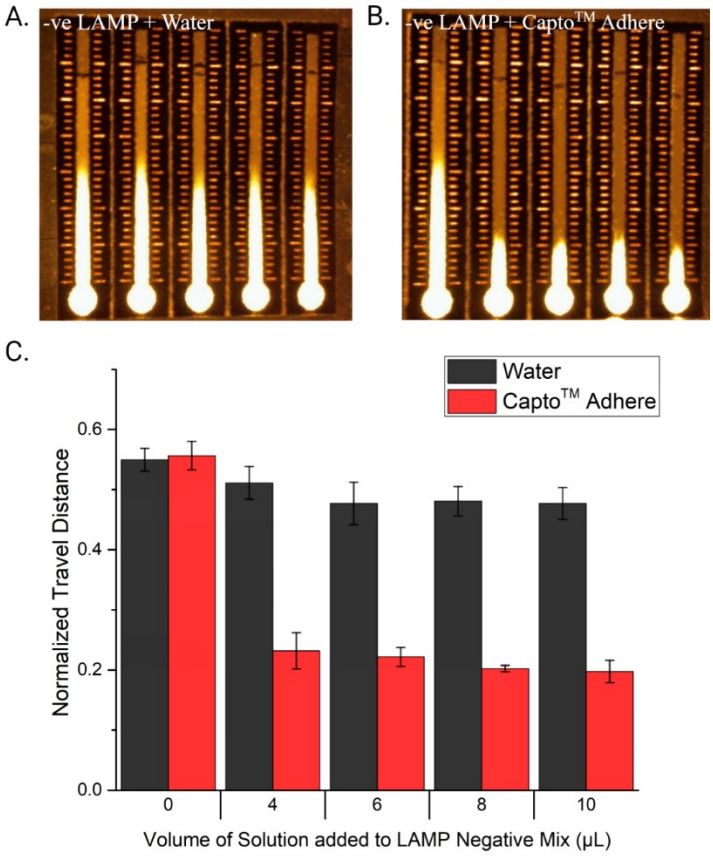

Figure S4. LAMP background and clean-Up. Representative fluorescent images of DBD substrates after exposure to 10 µL aliquots of amplified -ve control LAMP products mixed with various volumes (left-to-right, 0, 4, 6, 8, 10 µL) of (A) water or (B) a suspension of Capto™ Adhere beads. **C.** Plot of normalized travel distance (d_n) after exposure to volumes of water (black) or (B) a suspension Capto™ Adhere beads (red). Error bars represent ±1 std. deviation of normalized travel distance for n=3 replicates per condition.

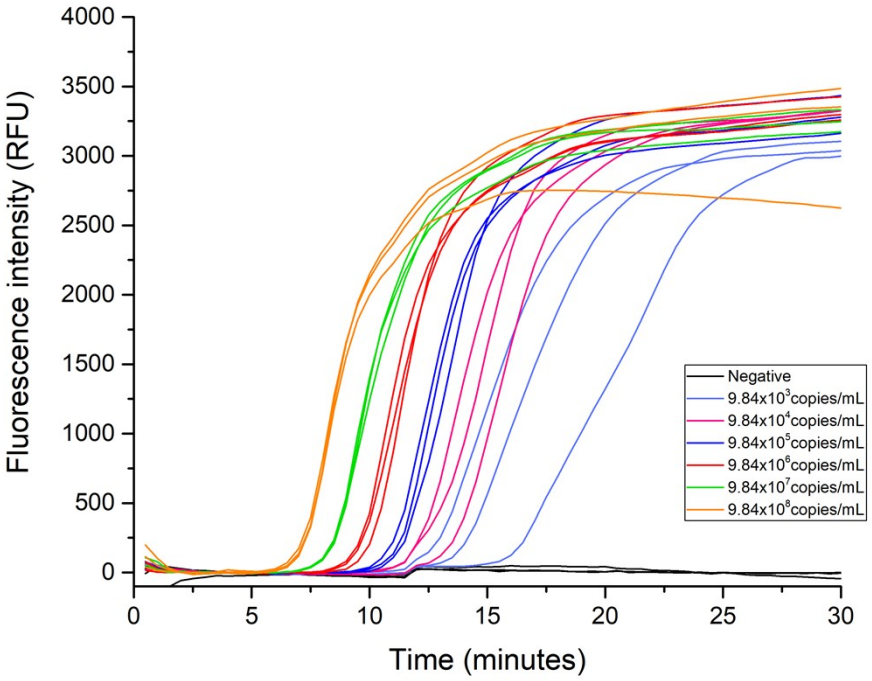

Figure S5. Quantitative LAMP Analysis of SARS-CoV-2 Samples. Plots of fluorescence intensity as a function of amplification time for a dilution series of extracted SARS-CoV-2 viral RNA [0 (black), 9.84×10^3 (light blue), 9.84×10^4 (pink), 9.84×10^5 (dark blue), 9.84×10^6 (red), 9.84×10^7 (green), and 9.84×10^8 (yellow) copies/mL], with three technical replicates per concentration.

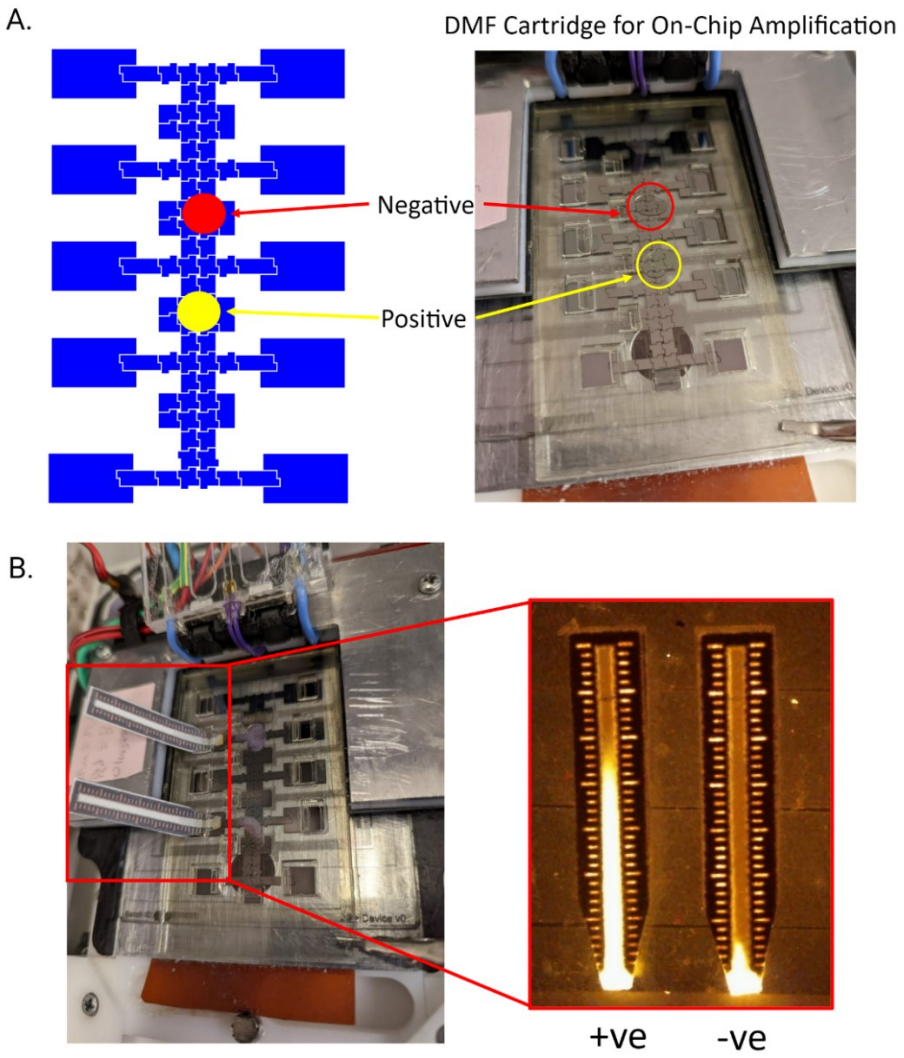

Figure S6. Automated DMF-LAMP-DBD. A. Schematic of DMF device electrode layout (left) and picture of DMF cartridge (right) used for automated LAMP amplification and cleanup, with negative and positive samples highlighted in red and yellow, respectively. **B.** Picture of cartridge with DBD substrates, with fluorescent image (inset) of substrates exposed to +ve (4.6×10^4 PFU/mL) and -ve (0 PFU/mL) samples of SARS-CoV-2 spiked into saliva.

Table S1. Concentrations of SARS-CoV-2 genome (± 1 std. dev. from n=3 samples tested per condition) measured by qPCR after spiking into human saliva.

Spiked viral load (PFU/mL) in saliva	N2 gene concentration determined by qPCR (copies/mL)
0	0
10	$(4.88 \pm 0.78) \times 10^4$
4.6×10^4	$(2.20 \pm 0.29) \times 10^8$